Increased lectin-like oxidized LDL receptor-1 expression in the placentas of women with intrahepatic cholestasis during pregnancy

Y. Yin, Q.Y. Zhu, S.J. Ren, D.M. Wang

Department of Obstetrics, First Affiliated Hospital of Xinjiang Medical University, Urumqi (China)

Summary

Background: Intrahepatic cholestasis of pregnancy (ICP) is a reversible cholestatic liver disease of undefined etiology and pathogenesis. It is likely associated with dyslipidemia. However, the explanation for the role of dyslipidemia is not clear. We hypothesized that an increased oxidized low-density lipoprotein (oxLDL) and lectin-like oxidized LDL receptor-1 (LOX-1) may play a crucial role in the development of the disease. Thus, the aim of this study was to investigate the plasma oxLDL level and LOX-1 expression in placentas from women with ICP. *Methodology:* The plasma oxLDL level and LOX-1 expression were detected in 94 intrahepatic cholestasis of pregnancy patients (ICP group) and 94 healthy pregnant women (control group). The placental LOX-1expression was detected by Western blotting; the plasma oxLDL was measured by enzyme-linked immunosorbent assay. *Results:* The placental LOX-1 expression in the ICP group was higher than that in the control group ($p < 0.05$), whereas the plasma oxLDL did not differ significantly between the patients with ICP and healthy pregnant women. *Conclusions:* LOX-1 may play a crucial role in the pathophysiological processes of ICP caused by over-apoptosis of trophocytes. Moreover, LOX-1 could be a potential target for therapeutic intervention.

Key words: Cholestasis; Pregnancy; OxLDL, LOX-1.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is the most common liver disorder in pregnancy that adversely affects maternal well being and fetal outcome. The etiology of ICP is complex and not fully understood, but it is likely associated with dyslipidemia [1]. However, no explanation for the role of dyslipidemia involvement in ICP has been offered. It is our aim here to supply the missing explanation. Small dense low density lipoprotein (LDL) was more susceptible to oxidation, resulting in the generation of oxidized LDL (oxLDL) [2]. Oxidative modification of LDL alters its biological properties, resulting in stronger cytotoxicity, so that it damages vascular endothelial cells and induces cell over-apoptosis. OxLDL can bind to the lectin-like oxidized LDL receptor-1 (LOX-1) on endothelial cells. LOX-1 is a type II membrane protein cell surface receptor identified in endothelial cells, vascular smooth muscle cells, and monocyte macrophages. Recent studies have reported that oxLDL and LOX-1were strongly related to various cell apoptoses [3]. However, no study has shown whether oxLDL and LOX-1 are associated with ICP. We hypothesized that there is an increased plasma oxLDL level and LOX-1 expression in placentas of women with ICP. We further hypothesized that these factors would contribute to over-apoptosis of trophocytes. Thus, the aim of this study was to investigate plasma oxLDL levels and LOX-1 expression in placentas from women with ICP.

Revised manuscript accepted for publication July 30, 2011

Material and Methods

Participants

Ninety-four women with ICP and 94 age- and gestational-week-matched healthy pregnant women (controls) were recruited prospectively from the patients in our hospital from January 2009 to April 2010. This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China). It was conducted according to the standards of the Declaration of Helsinki. Written informed consent was obtained from all participants. The clinical diagnosis of ICP was confirmed using values as reported [4]. On detection of abnormal liver function, possible alternative causes of liver disease were sought by testing for Epstein Barr virus, cytomegalovirus, hepatitis A, hepatitis B, and hepatitis C serology and by performing liver ultrasonography. Patients with hypertension, diabetes, nephritis, immune system disease and hyperthyreosis causing dyslipidemia were excluded. The characteristics of these subjects are described in Table 1.

Clinical assessments

Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Blood samples were obtained under fasting conditions from the median cubital vein and immediately centrifuged at 2000 g for 20 min and then stored at –80°C. Plasma lipid profiles and liver function were tested by a selective inhibition colorimetric assay using a direct double precipitation method (ABX Diagnostics, Montpellier, France). The measurement of plasma OxLDL was done by an enzyme-linked immunosorbent assay (ELISA) method [5] (OxLDL, ELISA Kit, USA).

Placental LOX-1 expression

Placenta biopsies were obtained within 30 min after delivery and then snap frozen in liquid nitrogen and stored at –80°C. Western blotting was for the detection of LOX-1. The cells were

lysed by a 4°C pretreated cell lysis solution, and the total protein level was determined by the Comassie blue method. The samples with 30 μg total protein each were applied for SDS-PAGE and were electro-transferred to the nitrocellular membrane (NC) membrane. In addition, 5% lipid-free milk powder was used for blocking for two hours. Anti-rat goat LOX-1 (1:200) was added, and the membrane was incubated at 4°C overnight. After washing, the anti-goat and anti-rabbit IgG antibody linked with horseradish peroxidase (HRP) were added for incubation for one hour, and the exposed film was later developed. Using β-actin as the internal control, the relative protein expression levels were determined quantitatively using an imaging analyzing system to scan the absorbance of specific bands.

Statistical analysis

Values are expressed as means ± SD. Comparison of two groups was conducted using the Student's *t*-test. All statistical analyses were completed using SPSS software version 16.0 (SPSS, Chicago, IL, USA); statistical significance was established as $p < 0.05$.

Table 1. — *Clinical characteristics and serum oxLDL in controls and ICP patients*.

Characteristic	ICP (n = 94)	Control (n = 94)
Age (years)	29 ± 4	28 ± 3
Gestational weeks	37.4 ± 3.7	38 ± 1.6
Body mass index (kg/m^2)	28.6 ± 3.3	27.9 ± 3.6
Ox-LDL (ng/l)	24.77 ± 10.90	22.87 ± 10.84
*TC (mmol/l)	6.51 ± 0.72	5.05 ± 1.19
*TG (mmol/l)	3.11 ± 0.19	2.32 ± 0.28
*LDL-C (mmol/l)	3.69 ± 1.47	3.24 ± 0.85
*HDL-C (mmol/l)	1.53 ± 0.18	2.23 ± 0.12
*DBIL (μmol/l)	16. 7 ± 9. 4	5. 8 ± 2.5
*TBIL (μmol/l)	26. 8 ± 11. 3	14. 4 ± 4. 5
*ALT (U/l)	167. 0 ± 54. 0	38.3 ± 12. 0
*AST (U/l)	133. 0 ± 41. 0	31. 0 ± 13. 0
*γ-GT (U/l)	78. 3 ± 22. 4	41. 6 ± 16. 8
*TBA (μmol/l)	45. 7 ± 27. 8	12. 3 ± 5. 1

Values presented are mean ± SD.
TC = total cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density; Ox-LDL = oxidized low-density lipoprotein; DBIL = direct bilirubin TBIL = total bilirubin; ALT = alanine aminotransferase; AST = aspartate aminotransferase γ-GT = γ-glutamyl transpeptidase; TBA = total bile acid.
*$p < 0.05$ versus controls.

Results

Clinical characteristics

In the ICP group, the mean maternal age was 29 ± 4 years and the median gestation week was 37.4 ± 3.7 weeks (range 32-40 weeks). Compared to the control group (28 ± 3 years, 38 ± 1.6 weeks), there were no significant differences (Table 1). Plasma lipid profiles and liver function of ICP women were significantly greater than for the control group ($p < 0.05$). The level of plasma oxLDL in the ICP group was slightly higher than the control group (Table 1), but there was no significant difference between them.

Placentas LOX-1 expression

LOX-1 expression was significantly increased in placentas from women with ICP (1.3898 ± 0.2961 pg/ml; $p < 0.05$) in comparison with the control group (0.9726 ± 0.2168 pg/ml) (Figure 1).

Discussion

Intrahepatic cholestasis in pregnancy (ICP) is a pregnancy-specific liver disorder characterized by maternal pruritus in the third trimester, raised serum bile acids and increased rates of adverse fetal outcomes. The etiology of ICP is complex and not fully understood, but it is likely associated with dyslipidemia, which may contribute to the pathogenesis of the disease. The elevation of LDL cholesterol and reduction of high-density lipoprotein cholesterol before clinical diagnosis may prove to be a useful biomarker for the early identification of ICP [1]. LDLs are more susceptible to oxidation, and oxidative modification of LDL alters its biological properties, resulting in chemotaxis of monocytes or T lymphocytes in addition to the modulation of growth factors and cytokine production from endothelial cells, smooth muscle cells, and macrophages [6]. Stimulation of the endogenous suicide cell death pathway by oxLDL in endothelial cells may be one cause of endothelial injury. In vitro studies have also demonstrated that oxLDL has cytotoxic effects on endothelial cells [7]. One study demonstrates that oxLDL induced dysfunction of the mitochondrial membrane potential, leading to cytochrome C release into the cytosol, and thereby stimulated apoptosis of human endothelial cells [8]. We hypothesized that there is an increased plasma oxLDL level and LOX-1 expression in placentas from women with ICP. We further hypothesized that these factors would contribute to the over-apoptosis of trophocytes. To our knowledge, however, there has been no report in the literature about oxLDL and LOX-1 in ICP. While our findings showed that there was no significant difference of plasma oxLDL level between ICP and control groups the placental LOX-1 expression in ICP was significantly higher than in the healthy pregnant women ($p < 0.05$).

It is envisioned that numerous factors will affect oxidized levels, such as oxidative stress, lipid content of LDL, LDL concentrations, conditions of the vascular wall, BMI, and clearance efficiency, to name a few [9]. However for patients with ICP, it is likely that oxLDL levels will show a relation to a higher level of total bilirubin (TBIL), because bilirubin is a physiological reductant with antioxidant activities. Its inhibitory action on the radical-mediated oxidation of LDL and plasma lipids has been investigated and the results showed that bilirubin inhibits oxidation of LDL lipids initiated within the lipoprotein core indicating that this activity is mediated by interaction of the pigment with LDL a-tocopherol [10]. An increased level of TBIL is one of the characteristics of ICP. The results of our study showed that the level of TBIL in the ICP group was higher than that in th control group ($p < 0.05$). Because of this multifactorial

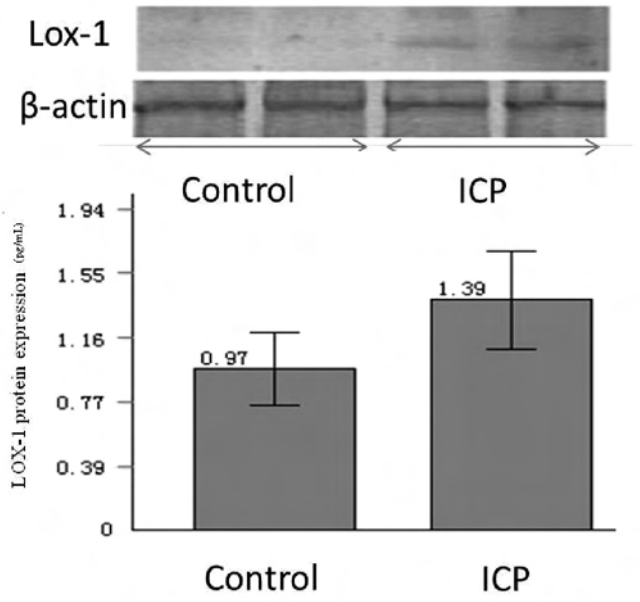

Figure 1. — Value of LOX-1 protein expression from placentas of ICP and control groups; a significant difference ($p < 0.05$) was observed.

regulation, oxLDL may reflect the combined effect of these numerous factors through additive as well as synergistic combination effects, although it may be difficult to delineate the individual contribution of each of these factors on the oxidative state. Therefore, it is difficult to deny that oxLDL levels represent a risk marker of ICP. Further investigations should be directed toward establishing the clinical importance of this marker in various stages of the progression of ICP.

LOX-1 is a type II single-transmembrane protein and a receptor for oxLDL expressed in vascular endothelial cells, macrophages, and vascular smooth muscle cells (VSMCs) in vitro and in vivo [11]. These results suggest that LOX-1 may mediate some of the pathophysiological consequences induced by oxLDL in various cell types. Northern blot analyses of various tissues revealed that aortic intima and vascular-rich organs, such as the placenta, lungs, brain and liver, express LOX-1 in vivo in physiological conditions [12]. LOX-1 shows a strong activity in binding, internalizing, and proteolytically degrading oxLDL. Up-regulation of LOX-1 could lead to the enhanced uptake and accumulation of oxLDL in the arterial walls, which could have deleterious effects by inducing and maintaining oxidative stress that may subsequently lead to endothelial cell dysfunction/apoptosis. A study was undertaken to determine the expression of LOX-1 in human renal biopsy specimens by an in situ hybridization technique and to clarify the relationship between the expression level of LOX-1 and clinical and histological features. The results suggest that the overexpression LOX-1 was closely related to the renal injury [13]. Our study demonstrates that LOX-l expression of placentas was higher in the ICP group than that in the control group ($p < 0.05$). Accordingly, it is believed that placental LOX-l may be a potential key marker for ICP. LOX-1 promoter expression can be induced by oxLDL, fluid shear stress, Ang II, proinflammatory cytokines, lipopolysaccharide, phorbol 12-myristate 13-acetate (PMA), oxidants, heparin-binding epidermal growth factor (HB-EGF) and others [14]. Nevertheless, the mechanism of placental over-expression of LOX-1 in ICP is uncertain.

LOX-1 may mediate over-apoptosis in VSMCs of placentas of ICP. Apoptosis is regulated by various apoptosis-related proteins. Bcl-2 acts as an anti-death factor preventing the release of cytochrome *c* (cyt c) and other apoptogenic factors from the mitochondria [15]. In contrast, Bax reduces mitochondrial membrane potential and thereby causes cyt *c* release and caspase activation, leading to apoptosis. Some experimental evidence has suggested that LOX-1 may mediate oxLDL-induced apoptosis through an increase in the Bax to Bcl-2 ratio in vivo [16]. Our previous study indicated that the placental dysfunction of ICP may be closely related to apoptosis, abnormal over-expression of p53, Bax and low expression of Bcl-2 on placental tissue as the main reason for placental over-apoptosis [17]. Therefore, LOX-1 may play a crucial role in the pathophysiological processes of ICP caused by over-apoptosis of trophocytes. Moreover, as over-apoptosis of placental trophocytes is related to the LOX-1 pathway, LOX-1 could be a potential target for therapeutic intervention.

References

[1] Dann A.T., Kenyon A.P., Wierzbicki A.S., Seed P.T., Shennan A.H., Tribe R.M.: "Plasma lipid profiles of women with intrahepatic cholestasis of pregnancy". *Obstet. Gynecol.,* 2006, *107,* 106.

[2] Wakatsuki A., Ikenoue N., Okatani Y., Shinohara K., Fukaya T.: "Lipoprotein particles in preeclampsia: susceptibility to oxidative modification". *Obstet. Gynecol*., 2000, *96,* 55.

[3] Salvayre R., Auge N., Benoist H., Negre-Salvayre A.: "Oxidized low-density lipoprotein-induced apoptosis". *Biochim. Biophys. Acta*, 2002, *1585*, 213.

[4] Dann A.T., Kenyon A.P., Seed P.T., Poston L., Shennan A.H., Tribe R.M.: "Glutathione-S-transferase and liver function in intrahepatic cholestasis of pregnancy and pruritus gravidarum". *Hepatology,* 2004, *40*, 1406.

[5] Holvoet P., Harris T.B., Tracy R.P,. Verhamme P., Newman A.B., Rubin S.M. *et al*.: "Association of high coronary heart disease risk status with circulating oxidized LDL in the well-functioning elderly: findings from the Health, Aging, and Body Composition study". *Arterioscler. Thromb. Vasc. Biol*., 2003, *23,* 1444.

[6] Deckert V., Persegol L., Viens L., Lizard G., Athias A., Lallemant C. *et al*.: "Inhibitors of arterial relaxation among components of human oxidized low-density lipoproteins: cholesterol derivatives oxidized in position 7 are potent inhibitors of endothelium-dependent relaxation". *Circulation,* 1997, 95, 723.

[7] Juckett M.B., Balla J., Balla G., Jessurun J., Jacob H.S., Vercellotti G.M.: "Ferritin protects endothelial cells from oxidized low density lipoprotein in vitro". *Pathol*., 1995, *47*, 782.

[8] Walter D.H., Haendeler J., Galle J., Zeiher A.M., Dimmeler S.: "Cyclosporin A inhibits apoptosis of human endothelial cells by preventing release of cytochrome C from mitochondria". *Circulation,* 1998, *98,* 1153.

[9] Toshima S., Hasegawa A., Kurabayashi M., Itabe H., Takano T., Sugano J. *et al*.: "Circulating oxidizd low density lipoprotein levels. A biochemical risk marker for coronary heart disease". *Arterioseler. Thromb. Vase. Biol.,* 2000, *20,* 2243.

[10] Neuzil J., Stocker R.: "Free and albumin-bound bilirubin are efficient co-antioxidants for alpha-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation". *Bio. Chem.,* 1994, *269,* 16712.
[11] Minami M., Kume N., Kataoka H., Morimoto M., Hayashida K., Sawamura T. *et al*.: "Transforming growth factor-(1) increases the expression of lectin-like oxidized low-density lipoprotein receptor-1". *Biochem. Biophys Res. Commun.,* 2000, *272,* 357.
[12] Sawamura T., Kume N., Aoyama T., Moriwaki H., Hoshikawa H., Aiba Y. *et al*.: "An endothelial receptor for oxidized low-density lipoprotein". *Nature*, 1997, *386,* 73.
[13] Wilson S.H., Chade A.R., Feldstein A., Sawamura T., Napoli C., Lerman A., Lerman L.O.: "Lipid-lowering independent effects of simvastatin on the kidney in experimental hypercholesterolaemia". *Nephrol. Dial. Transplant.,* 2003, *18,* 703.
[14] Navarra T., Del Turco S., Berti S., Basta G.: "The Lectin-like oxidized low-density lipoprotein receptor-1 and its soluble form: cardiovascular implications". *Atheroscler. Thromb.,* 2010, *17*, 317.
[15] Kluck R.M., Bossy-Wetzel E., Green D.R., Newmeyer D.D.: "The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis". *Science,* 1997, *275*, 1132.
[16] Salvayre R., Auge N., Benoist H., Negre-Salvayre A.: "Oxidized low-density lipoprotein-induced apoptosis". *Biochem. Biophys. Acta,* 2002, *1585,* 213.
[17] Wang D.M., Zhu Q.Y., Ding L.: "Relationship between p 53, Bax and bcl-2 expression and cell apoptosis in intrahepatic cholestasis of pregnancy". *Chin. J. Obstet. Gynecol.,* 2003, *38,* 5.

Address reprint requests to:
D.M. WANG, M.D., Ph.D.
Department of Obstetrics
First Affiliated Hospital of Xinjiang
Medical University
Urumqi (China)
e-mail: karenwdm000@126.com